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(54) Title: REDUCING AGENT FOR REDUCTIVE ALKYLATION OF GLYCOPEPTIDE ANTIBIOTICS

(57) Abstract

This invention is concerned with improved processes for reductive alkylation of glycopeptide antibiotics, the improvement residing in employing pyridine–borane as reducing agent.

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REDUCING AGENT FOR REDUCTIVE ALKYLATION OF GLYCOPEPTIDE ANTIBIOTICS

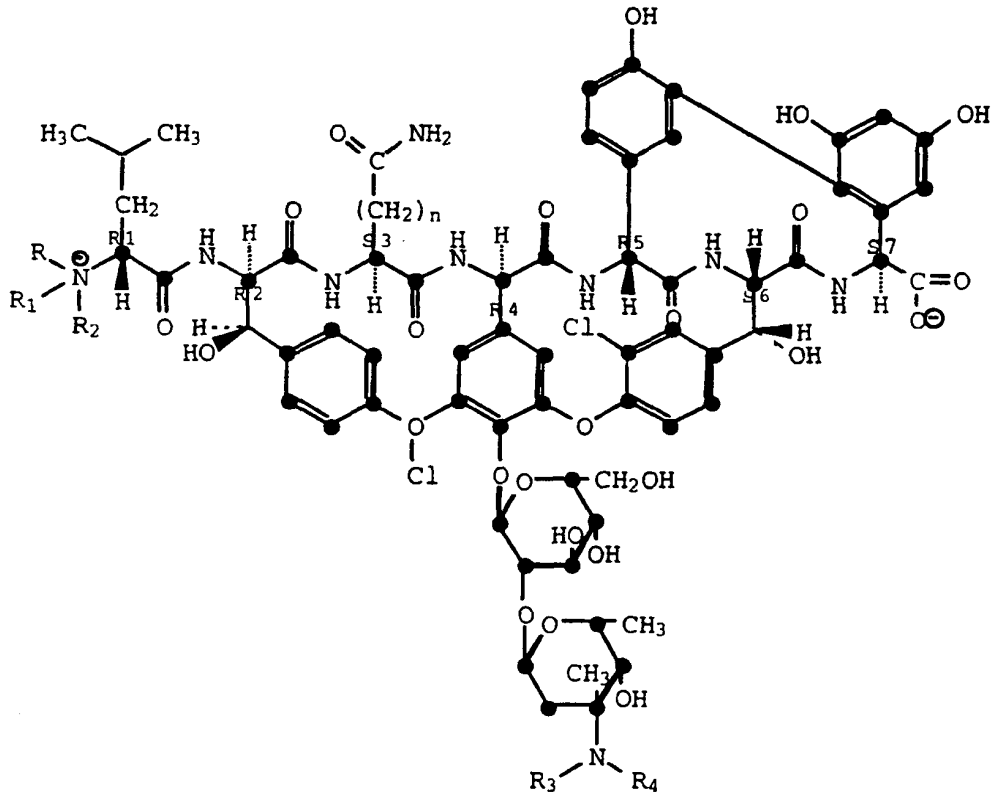
The present invention is directed to improved methods for reductively alkylating glycopeptide antibiotics, the improvement residing in the use of pyridine·borane complex as the reducing agent.

The present invention relates to reductive alkylation of glycopeptide antibiotics.

The glycopeptide antibiotics are a large class of substances either produced by microorganisms, or produced by microorganisms and thereafter subsequently modified in part. Two of these, vancomycin and teicoplanin, are sold as antibacterial products, but many others have been discovered and are being considered for development, especially since the emergence in the late 1980s of resistance to various antibiotics, including the glycopeptides themselves. The entire class of glycopeptide antibiotics is well described in "Glycopeptide Antibiotics", edited by Ramakrishnan Nagarajan (Marcel Dekker, Inc., New York, 1994). Among the more recently discovered glycopeptides are those known as A82846A (also called ereomomycin), A82846B (also known as chloroorienticin A), A82846C (also known as orienticin C), and orienticin A. The present invention is preferred for use with vancomycin type glycopeptide antibiotics, including vancomycin, A82846A, A82846B, A82846C, and orienticin A. The invention is especially preferred for use with A82846B.

Many modifications of naturally-occurring glycopeptides have been made. Among the modifications are reductive alkylations of reactive amine(s) in glycopeptides. See, for example, U.S. 4,698,327 describing reductive alkylations of vancomycin, and EPO 435 503 A1 and EPO 667 353 A1, both of which describe reductive alkylations of a variety of glycopeptides including vancomycin, A82846A, A82846B, A82846C, and orienticin A. These references describe reductive alkylations which introduce into the parent glycopeptides a great variety of alkyl groups.

4,698,327 describes alkylated vancomycin compounds of the formula:



wherein

R is hydrogen or methyl;

n is 1 or 2; and

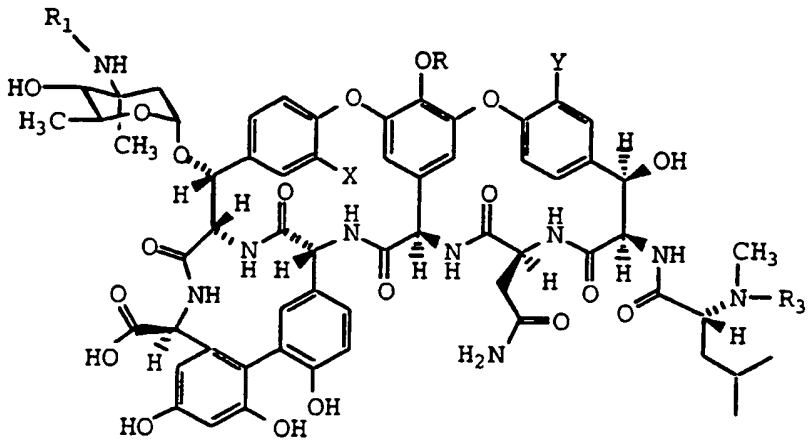
R_1 is hydrogen or methyl;

R_2 and R_3, independently, are hydrogen or a group of the formula: R_6R_7CH-;

R_6 and R_7 are independently R_5, R_5-(C_1-C_5-alkyl) or R_5-(C_2-C_5-alkenyl);

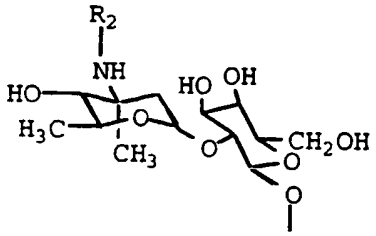
R_5 is hydrogen, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl, C_1-C_4 alkoxy, C_3-C_{10}-cycloalkyl, C_5-C_{12}-cycloalkenyl, phenyl, naphthyl, indenyl, tetralinyl, decalinyl, adamantyl, a monocyclic heterocyclic ring system comprising 3 to 8 atoms in the ring or a bicyclic heterocyclic ring system comprising 6 to 11 atoms, provided that at least one atom of the ring system is carbon and at least one atom of the ring system is a heteroatom selected from O, N, and S, and R_5 may be substituted with one or more hydroxy, nitro, C_1-C_{10}-alkoxy, C_1-C_{10}-alkyl, phenyl, C_1-C_6-alkylthio, nitrile, halo, C_2-C_4-acylamino, amino, C_1-C_4-dialkylamino groups; and R_4 is hydrogen, provided that: (1) at least one of R_2 and R_3 must be other than hydrogen; (2) when n is 2, R must be hydrogen; (3) when R is methyl and R_3 is hydrogen, R_2 cannot be methyl and (4) when R and R_1 are both methyl, then R_2 is hydrogen or methyl and n is 1.

EPO 435 503 A1 is directed to alkylated and acylated glycopeptides of the formula:

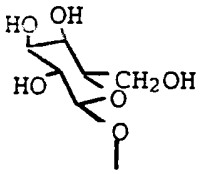


wherein:

R is hydrogen or a (4-epi-vancosaminyl)-O-glucosyl group of formula



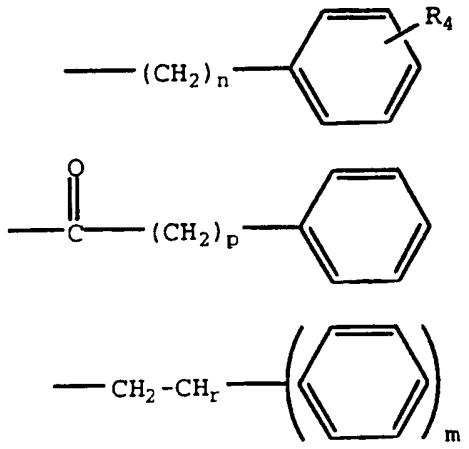
or the glucosyl group of formula



X is hydrogen or chloro;

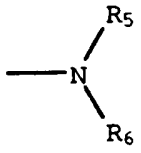
Y is hydrogen or chloro;

R_1, R_2, and R_3 are independently hydrogen; C_1-C_{12} alkyl; C_2-C_9 alkanoyl; or a group of formula



n is 1 to 3;

R_4 is hydrogen, halo, C_1-C_8 alkyl, C_1-C_8 alkoxy, or a group of formula



R_5 and R_6 are independently hydrogen or C_1-C_3 alkyl;

p is 0 to 2;

m is 2 or 3, and r = 3 - m; provided that, where R is a (4-epi-vancosaminyl)-O-glucosyl group, R_1, R_2, and R_3 are not all hydrogen, and where R is hydrogen or a glucosyl group, R_1 and R_3 are not both hydrogen.

Where R is (4-epi-vancosaminyl)-O-glucosyl, the glycopeptides so defined are

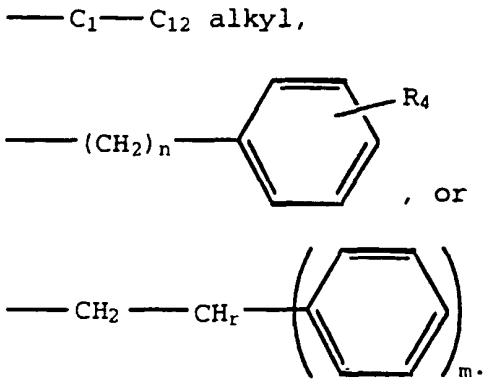
X = H, Y = Cl, A82846A

X = Y = Cl, A82846B

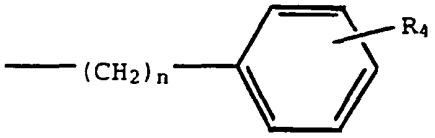
X = Y = H, A82846C

X = Cl, Y = H, orienticin A.

Thus, EPO 435 503 A1 describes alkyl derivatives of A82846A, A82846B, A82846C, and orienticin A wherein the alkyl group is

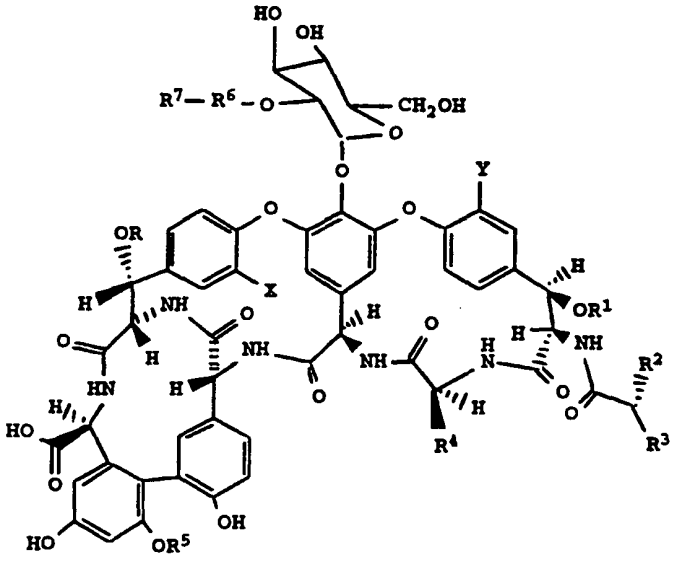


Preferred groups are C_8-C_{12} alkyl and groups of the formula



wherein R_4 is hydrogen, halo, C_1-C_8 alkyl, or C_1-C_8 alkoxy.

EPO 667 353 A1 describes alkylated glycopeptide antibiotics of the formula



wherein:

X and Y are each independently hydrogen or chloro;

R is hydrogen, 4-*epi*-vancosaminyl, actinosaminyl, or ristosaminyl;

R^1 is hydrogen, or mannose;

R^2 is $-NH_2$, $-NHCH_3$, or $-N(CH_3)_2$;

R^3 is $-CH_2CH(CH_3)_2$, [*p*-OH, *m*-Cl]phenyl, *p*-rhamnose-phenyl, or [*p*-rhamnose-galactose]phenyl, [*p*-galactose-galactose]phenyl, [*p*-CH_3O-rhamnose]phenyl;

R^4 is $-CH_2(CO)NH_2$, benzyl, [*p*-OH]phenyl, or [*p*-OH, m-Cl]phenyl;

R^5 is hydrogen, or mannose;

R^6 is vancosaminyl, 4-*epi*-vancosaminyl, L-acosaminyl, L-ristosaminyl, or L-actinosaminyl;

R^7 is (C_2-C_{16})alkenyl, (C_2-C_{12})alkynyl, $(C_1-C_{12}$ alkyl$)-R_8$, $(C_1-C_{12}$ alkyl$)$-halo, $(C_2-C_6$ alkenyl$)-R_8$, $(C_2-C_6$ alkynyl$)-R_8$, $(C_1-C_{12}$ alkyl$)-O-R_8$, and is attached to the amino group of R^6;

R^8 is selected from the group consisting of:

a) multicyclic aryl unsubstituted or substituted with one or more substituents independently selected from the group consisting of:

(i) hydroxy,

(ii) halo,

(iii) nitro,

(iv) (C_1-C_6)alkyl,

(v) (C_2-C_6)alkenyl,

(vi) (C_2-C_6)alkynyl,

(vii) (C_1-C_6)alkoxy,

(viii) halo-(C_1-C_6)alkyl,

(ix) halo-(C_1-C_6)alkoxy,

(x) carbo-(C_1-C_6)alkoxy,

(xi) carbobenzyloxy,

(xii) carbobenzyloxy substituted with (C_1-C_6)alkyl, (C_1-C_6)alkoxy, halo, or nitro,

(xiii) a group of the formula $-S(O)_{n'}-R^9$, wherein n' is 0-2 and R^9 is (C_1-C_6)alkyl, phenyl, or phenyl substituted with (C_1-C_6)alkyl, (C_1-C_6)alkoxy, halo, or nitro, and

(xiv) a group of the formula $-C(O)N(R^{10})_2$ wherein each R^{10} substituent is independently hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, phenyl, or phenyl substituted with (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, halo, or nitro;

b) heteroaryl unsubstituted or substituted with one or more substituents independently selected from the group consisting of:

(i) halo,

(ii) (C_1-C_6)alkyl,

(iii) (C_1-C_6)alkoxy,

(iv) halo-(C_1-C_6)alkyl,

(v) halo-(C_1-C_6)alkoxy,

(vi) phenyl,

(vii) thiophenyl,

(viii) phenyl substituted with halo, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, (C_1-C_6)alkoxy, or nitro,

(ix) carbo-(C_1-C_6)alkoxy,

(x) carbobenzyloxy,

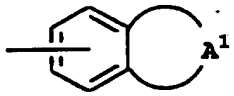
(xi) carbobenzyloxy substituted with (C_1-C_6)alkyl, (C_1-C_6) alkoxy, halo, or nitro,

(xii) a group of the formula $-S(O)_n-R^9$, as defined above,

(xiii) a group of the formula $-C(O)N(R^{10})_2$ as defined above, and

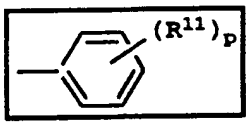
(xiv) thienyl;

c) a group of the formula:



wherein A^1 is $-OC(A^2)_2-C(A^2)_2-O-$, $-O-C(A^2)_2-O-$, $-C(A^2)_2-O-$, or $-C(A^2)_2-C(A^2)_2-C(A^2)_2-C(A^2)_2-$, and each A^2 substituent is independently selected from hydrogen, (C_1-C_6)-alkyl, (C_1-C_6)alkoxy, and (C_4-C_{10})cycloalkyl;

d) a group of the formula:



wherein p is from 1 to 5; and

R^{11} is independently selected from the group consisting of:

(i) hydrogen,

(ii) nitro,

(iii) hydroxy,

(iv) halo,

(v) (C_1-C_8)alkyl,

(vi) (C_1-C_8)alkoxy,

(vii) (C_9-C_{12})alkyl,

(viii)(C_2-C_9)alkynyl,

(ix) (C_9-C_{12})alkoxy,

(x) (C_1-C_3)alkoxy substituted with (C_1-C_3)alkoxy, hydroxy, halo(C_1-C_3)alkoxy, or (C_1-C_4)alkylthio,

(xi) (C_2-C_5)alkenyloxy,

(xii) (C_2-C_{13})alkynyloxy

(xiii) halo-(C_1-C_6)alkyl,

(xiv) halo-(C_1-C_6)alkoxy,

(xv) (C_2-C_6)alkylthio,

(xvi) (C_2-C_{10})alkanoyloxy,

(xvii) carboxy-(C_2-C_4)alkenyl,

(xviii) (C_1-C_3)alkylsulfonyloxy,

(xix) carboxy-(C_1-C_3)alkyl,

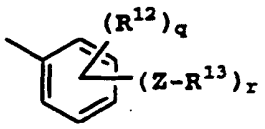
(xx) N-[di(C_1-C_3)-alkyl]amino-(C_1-C_3)alkoxy,

(xxi) cyano-(C_1-C_6)alkoxy, and

(xxii) diphenyl-(C_1-C_6)alkyl,

with the proviso that when R^{11} is (C_1-C_8)alkyl, (C_1-C_8)alkoxy, or halo, p must be greater or equal to 2, or when R^7 is (C_1-C_3 alkyl)-R^8 then R^{11} is not hydrogen, (C_1-C_8)alkyl, (C_1-C_8)alkoxy, or halo;

e) a group of the formula:



wherein q is 0 to 4;

R^{12} is independently selected from the group consisting of:

(i) halo,

(ii) nitro,

(iii) (C_1-C_6)alkyl,

(iv) (C_1-C_6)alkoxy,

(v) halo-(C_1-C_6)alkyl,

(vi) halo-(C_1-C_6)alkoxy, and

(vii) hydroxy, and

(vii) (C_1-C_6)thioalkyl;

r is 1 to 5; provided that the sum of q and r is no greater than 5;

Z is selected from the group consisting of:

(i) a single bond,

(ii) divalent (C_1-C_6)alkyl unsubstituted or substituted with hydroxy, (C_1-C_6)alkyl, or (C_1-C_6)alkoxy,

(iii) divalent (C_2-C_6)alkenyl,

(iv) divalent (C_2-C_6)alkynyl, or

(v) a group of the formula $-(C(R^{14})_2)_s-R^{15}-$ or $-R^{15}-(C(R^{14})_2)_s-$, wherein s is 0-6; wherein each R^{14} substituent is independently selected from hydrogen, (C_1-C_6)-alkyl, or (C_4-C_{10}) cycloalkyl; and R^{15} is selected from -

O-, -S-, -SO-, $-SO_2-$, $-SO_2-O-$, -C(O)-, -OC(O)-, -C(O)O-, -NH-, $-N(C_1-C_6$ alkyl)-, and -C(O)NH-, -NHC(O)-, N=N;

R^{13} is independently selected from the group consisting of:

(i) (C_4-C_{10})heterocyclyl,

(ii) heteroaryl,

(iii) (C_4-C_{10})cycloalkyl unsubstituted or substituted with (C_1-C_6)alkyl, or

(iv) phenyl unsubstituted or substituted with 1 to 5 substituents independently selected from: halo, hydroxy, nitro, (C_1-C_{10}) alkyl, (C_1-C_{10})alkoxy, halo-(C_1-C_3)alkoxy, halo-(C_1-C_3)alkyl, (C_1-C_3)alkoxyphenyl, phenyl, phenyl-(C_1-C_3)alkyl, (C_1-C_6)alkoxyphenyl, phenyl-(C_2-C_3)alkynyl, and (C_1-C_6)alkylphenyl;

f) (C_4-C_{10})cycloalkyl unsubstituted or substituted with one or more substituents independently selected from the group consisting of:

(i) (C_1-C_6)alkyl,

(ii) (C_1-C_6)alkoxy,

(iii) (C_2-C_6)alkenyl,

(iv) (C_2-C_6)alkynyl,

(v) (C_4-C_{10})cycloalkyl,

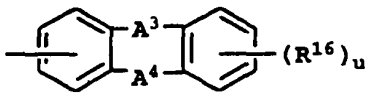
(vi) phenyl,

(vii) phenylthio,

(viii) phenyl substituted by nitro, halo, (C_1-C_6)alkanoyloxy, or carbocycloalkoxy, and

(ix) a group represented by the formula -Z-R^{13} wherein Z and R^{13} are as defined above; and

g) a group of the formula:



wherein

A^3 and A^4 are each independently selected from

(i) a bond,

(ii) -O-,

(iii) $-S(O)_t-$, wherein t is 0 to 2,

(iv) $-C(R^{17})_2-$, wherein each R^{17} substituent is independently selected from hydrogen, (C_1-C_6)alkyl, hydroxy, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, or both R^{17} substituents taken together are O,

(v) $-N(R^{18})_2-$, wherein each R^{18} substituent is independently selected from hydrogen; (C_1-C_6)alkyl; (C_2-C_6)alkenyl; (C_2-C_6)alkynyl; (C_4-C_{10})cycloalkyl; phenyl; phenyl substituted by nitro, halo, (C_1-C_6)alkanoyloxy; or both R^{18} substituents taken together are (C_4-C_{10})cycloalkyl;

R^{16} is R^{12} or R^{13} as defined above; and

u is 0-4.

In this reference, preferred glycopeptide antibiotics are A82846A, A82846B, A82846C, and orienticin A; preferred alkyls are those wherein R^7 is CH_2-R_8; and preferred R^8 moieties are those defined as groups "(d)" and "(e)".

The present invention can be utilized to make the alkylated glycopeptides described in these references. Preferred alkylated glycopeptides which can be prepared by the present process include the following:

N^4-n-octylA82846B

N^4-n-decylA82846B

N^4-benzylA82846B

N^4-(p-chlorobenzyl)A82846B

N^4-(p-bromobenzyl)A82846B

N^4-(p-propylbenzyl)A82846B

N^4-(p-isopropylbenzyl)A82846B

N^4-(p-butylbenzyl)A82846B

N^4-(p-isobutylbenzyl)A82846B

N^4-(p-pentylbenzyl)A82846B

N^4-(p-isohexylbenzyl)A82846B

N^4-(p-octylbenzyl)A82846B

N^4-(p-propoxybenzyl)A82846B

N^4-(p-isopropoxybenzyl)A82846B

N^4-(p-butoxybenzyl)A82846B

N^4-(p-tert-butoxybenzyl)A82846B

N^4-(p-pentyloxybenzyl)A82846B

N^4-(p-hexyloxybenzyl)A82846B

N^4-(o-hexyloxybenzyl)A82846B

N^4-(p-heptyloxybenzyl)A82846B

N^4-(p-octyloxybenzyl)A82846B

N^4-phenethylA82846B

N^4-(4-phenylbenzyl)A82846B

N^4-(4-(4-chlorophenyl)benzylA82846B

N^4-(4-(4-methylbenzyloxy)benzyl)A82846B

N^4-(4-(4-ethylbenzyloxy)benzyl)A82846B

N^4-(4-(4-chlorophenethyl)benzyl)A82846B

N^4-(4-(2-(4-methoxyphenyl)ethynyl)benzyl)A82846B.

The references noted above describe the reductive alkylation as comprising a first step, in which the glycopeptide is reacted with the respective aldehyde or ketone to form a Schiff's base, which in a second step is reduced to the desired alkylated product. In one variation of this procedure, EPO 667 353 A1 describes a process in which the reducing agent is added simultaneously with the glycopeptide and aldehyde or ketone.

The references suggest a strong preference for sodium cyanoborohydride as reducing agent. While sodium cyanoborohydride is a successful reagent for small scale use, its use in large scale production is less satisfactory. This is due to safety and environmental issues posed by the cyanide ion. Accordingly, sodium cyanoborohydride is less than an ideal reducing agent for larger scale reactions.

Reducing agents are legion, but many are unsatisfactory for the glycopeptides. One of the many reducing agents known for use in reductive alkylations is pyridine·borane (see J. Chem Soc, Perkin Trans. 1 (1984), pages 717-720, which is incorporated herein by reference). It has now been discovered that pyridine·borane is a uniquely acceptable reagent for alkylative reductions on glycopeptides, while presenting no safety or environmental hazards as is the case

with sodium cyanoborohydride. Furthermore, in a preferred embodiment, it has been discovered that portionwise addition of the pyridine·borane increases yields.

Thus, the present invention is directed to an improved process for reductively alkylating an amine-containing glycopeptide antibiotic, which process comprises reacting the glycopeptide antibiotic with an aldehyde or ketone in the presence of a reducing agent, wherein the improvement comprises employing pyridine·borane as reducing agent. In a preferred embodiment, the glycopeptide antibiotic, aldehyde or ketone, and a portion of the reducing agent are mixed together at the same time, and one or more additional portions of reducing agent are added thereafter.

In carrying out the present invention, standard conditions for reductive alkylations of glycopeptides are employed, other than the identity of the reducing agent and the preference for its portionwise addition. Thus, the glycopeptide and aldehyde or ketone are initially dissolved in a solvent which is at least predominantly methanol, and which is preferably only methanol. If only these reagents are supplied, some small amount of Schiff's base is produced, but the reaction equilibrium does not favor complete production of the Schiff's base. Addition of reducing agent shifts the equilibrium as Schiff's base is converted to alkylated product. As noted, EPO 667 353 A1 teaches a preference for simultaneous addition of the reducing agent with the glycopeptide antibiotic and aldehyde or ketone. Thus, in the present invention, the

glycopeptide, aldehyde or ketone, and pyridine·borane are added at essentially the same time.

Further, it has been discovered that when employing pyridine·borane as reducing agent, even simultaneous addition of glycopeptide antibiotic, aldehyde or ketone, and reducing agent leads to only modest yields and that such yields can be increased by portionwise addition of the pyridine·borane, with no more than a portion being added initially to the glycopeptide antibiotic and the aldehyde or ketone.

The exact number and timing of portions is not critical. The reaction is generally conducted over a period of time from 4 to 48 hours and preferably from 6 to 24 hours. In the preferred practice of the present invention, a first portion of pyridine·borane is added with the glycopeptide antibiotic and aldehyde or ketone, and the remainder of the pyridine·borane is added in one, two, or more subsequent portions. The ideal sequence of pyridine·borane addition appears to be five portions at 2 to 4 hour intervals (counting the initial addition as the first of the five). Devices can be employed to provide a continuous delivery of the pyridine·borane.

In another preferred embodiment of the invention, a source of soluble copper is supplied to the reaction mixture, initially converting the glycopeptide to a copper complex, which becomes the reactive entity. The use of copper confers regioselectivity of reaction in those glycopeptides having multiple reactive amines. For example,

in A82846B, the use of copper minimizes reaction on the N^1 (leucine) site and on the N^6 (monosaccharide) site, thereby providing higher yields of the product monoalkylated on the N^4 (disaccharide) amine.

The identity of the copper source is not critical, so long as it is at least partially soluble and does not negatively impact the pH. Suitable copper salts are cupric acetate, cupric trifluoroacetate, cupric cyclohexanebutyrate, cupric 2-ethylhexanoate, cuprous chloride, cupric chloride, and cupric bromide. A preferred source of copper is copper (II) acetate, most conveniently employed as the hydrate.

The reaction should be conducted at a pH of 6-8, and preferably at a pH of 6.3-7.0.

The amounts of reactants and reagents to be employed are not critical; amounts to maximize the yield of product will vary somewhat with the identity of the reactants. The reaction consumes the glycopeptide antibiotic and the aldehyde or ketone in equimolar amounts. A slight excess of the aldehyde or ketone, e.g., 1.3 to 1.7:1, is preferred. The amount of the glycopeptide antibiotic to be used must be corrected for its purity. The reaction consumes an equimolar amount of the pyridine·borane. A slight excess is preferable. The amount of soluble copper, if used, is important. The process first results in the formation of a 1:1 copper complex with the glycopeptide antibiotic. Therefore, the copper is preferably present in an amount

approximately equimolar with the glycopeptide antibiotic. However, amounts exceeding one molar equivalent are undesirable because excess copper decomposes the pyridine·borane.

Summarizing the foregoing, the ideal amounts to be employed are a ratio of:
glycopeptide:aldehyde or ketone:reducing agent:copper salt of:

1:1.3 to 1.7:1.5:0.9 to 1.

The concentration of the reactants in the solvent has some bearing on the process. Methanol volume relative to mass of glycopeptide antibiotic can vary from 50:1 to 500:1; a 100:1 dilution appears to be a useful, practical ratio, although higher dilutions may give slightly higher yields.

The temperature at which the process is carried out is important. Reaction mixtures in methanol boil at about 67°C., thereby setting the maximum temperature when employing straight methanol as the solvent. Higher temperatures are of course possible when employing mixtures of methanol or when operating under pressure. Lower temperatures can be tolerated, but preferably not lower than about 45°C. The ideal conditions depend upon whether or not copper is employed in the reaction. When copper is not added, it is preferred to conduct the reaction in straight methanol at temperatures of about 58-67°C. When employing copper, it is important to conduct the reaction at slightly lower temperatures of about 58-63°C; again, straight methanol is preferred.

Upon the completion of the reaction, the reaction mixture is preferably quenched, as by the addition of sodium borohydride. This reagent consumes residual aldehyde or ketone and thereby prevents further undesired reactions.

The product is isolated from the reaction mixture in conventional manner. When copper has been employed, the product is isolated from the reaction mixture as a copper complex of the alkylated glycopeptide. Isolation is achieved by concentration of the reaction mixture and precipitation of the complex by addition of an antisolvent such as ethyl acetate, acetone, 1-propanal, isopropyl alcohol, or preferably acetonitrile. The complex can be broken by aqueous treatment at pH ≥4, freeing the simple alkylated glycopeptide product, which can, if desired, be purified in conventional manner.

The following examples illustrate the present invention and will enable those skilled in the art to practice the same.

EXAMPLE 1

A82846B (0.50 g, 84.3% potency, 0.42 bg, 0.26 mmol) was stirred in 50 mL methanol and 4'-chloro-4-biphenylcarboxaldehyde (72 mg, 0.33 mmol) and pyridine·borane complex (0.033 mL, 0.33 mmol) were added. The mixture was heated at reflux for 6 hours before being cooled to ambient temperature. HPLC analysis of a reaction aliquot afforded a yield of 0.25 g (53.2%) of N^4-(4-(4-chlorophenyl)benzyl)A82846B.

EXAMPLE 2

(portionwise addition of pyridine·borane)

A82846B (0.50 g, 83.9 % potency, 0.26 mmol) and 4'-chloro-4-biphenylcarboxaldehyde (98 mg, 0.45 mmol) were stirred in 50 mL methanol and pyridine·borane complex (0.015 mL, 0.15 mmol) was added. The reaction mixture was heated at reflux for 4 hours and an additional aliquot of pyridine·borane complex (0.015 mL, 0.15 mmol) was added. After heating at reflux for 4 hours longer a final addition of pyridine·borane complex (0.015 mL, 0.15 mmol) was made. The reaction mixture was heated at reflux for another 20 hours. After cooling to ambient temperature HPLC analysis of a reaction aliquot afforded a yield of 0.27 g (58.0 %) of N^4-(4-(4-chlorophenyl)benzyl)A82846B.

EXAMPLE 3

(with copper)

A82846B (0.50 g, 84.3% potency, 0.42 bg, 0.26 mmol) was stirred in 50 mL methanol and cupric acetate (45 mg, 0.25 mmol) was added. After stirring at ambient temperature for 10 min, 4'-chloro-4-biphenylcarboxaldehyde (84 mg, 0.39 mmol) and pyridine·borane complex (0.039 mL, 0.39 mmol) were added. The mixture was heated at 57° C for 24 hours before being cooled to ambient temperature. HPLC analysis of a reaction aliquot afforded a yield of 0.34 g (72.3%) of N^4-(4-(4-chlorophenyl)benzyl)A82846B.

EXAMPLE 4

(with copper + portionwise addition of pyridine·borane)

A82846B (0.50 g, 76.3% potency, 0.38 bg, 0.24 mmol) and cupric acetate monohydrate (43 mg, 0.216 mmol) were stirred in 50 mL methanol and 4'-chloro-4-biphenylcarboxaldehyde (84.5 mg, 0.39 mmol) and pyridine·borane complex (0.011 mL, 0.11 mmol) were added. The mixture was heated at 63°C for 2 hours and an additional portion of pyridine·borane was added (0.01 mL, 0.1 mmol). After 2 hours more at 63°C a third portion of pyridine·borane (0.005 mL, 0.05 mmol) was added. A fourth portion of pyridine·borane (0.005 mL, 0.05 mmol) was added 2 hours later followed by a fifth portion of pyridine·borane (0.005 mL, 0.05 mmol) after another 5 hours at 63°C. The mixture was heated at 63°C for another 11 hours before being cooled to ambient temperature. HPLC analysis of a reaction aliquot afforded a yield of 0.34 g (79.2%) of N^4-(4-(4-chlorophenyl)benzyl)A82846B.

The reactions reported in Examples 1, 3, and 4 were also evaluated (1) for the amount of the remaining starting glycopeptide, (2) for the amount of products alkylated on amine sites other than the N^4-position, and (3) for the amount of multiply-alkylated products. The results are set forth in the following table and are expressed as a percentage relative to the intended product monoalkylated on the N^4-amine; yields of the intended product are actual yields as recited in the foregoing examples.

TABLE I

Ex. No.	% Mono-alkylated at N^4	% A82846B	% Mono-alkylated at N^6	% Mono-alkylated at N^1	% Di-alkylated at both N^4 and N^6	% Di-alkylated at both N^1 and N^4	% Tri-alkylated
1	53.2	47.6	9.9	1.3	21.7	7.8	1.8
3	72.3	17.8	2.1	0.7	6.2	2.5	0.4
4	79.2	9.2	1.4	0.3	7.4	3.4	0.3

These data show that portionwise addition of pyridine·borane, accompanied by the use of copper, maximizes yields of the product monoalkylated on N^4, while minimizing yields of other alkylated products.

EXAMPLE 5

(with copper + portionwise addition of pyridine·borane by syringe pump)

A82846B (0.50 g, 83.4 % potency, 0.26 mmol) and cupric acetate (47 mg, 0.26 mmol) were stirred in 50 mL methanol and 4'-chloro-4-biphenylcarboxaldehyde (98 mg, 0.45 mmol) and pyridine·borane complex (0.015 mL, 0.15 mmol) were added. The reaction mixture was heated at 63°C for 2 hours. Additional pyridine·borane complex (0.03 mL, 0.30 mmol) in 2 mL methanol was added to the reaction mixture at a rate of 400 uL/hour using a syringe pump. The temperature was maintained at 63°C during the addition. After the addition was complete, heating was continued to afford a total reaction time of 24 hours. After cooling to ambient

temperature, HPLC analysis of a reaction aliquot afforded a yield of 0.35 g (74.3 %) of N^4-(4-(4-chlorophenyl)benzyl)A82846B.

The following HPLC System was used for *in situ* reaction monitoring and yield calculation: HPLC system Waters 600E with HP3395 integrator and Applied Biosystems 757 detector set at 230 nm, sensitivity 0.1 absorption units, 1 sec. filter rise time. Column: DuPont Zorbax SB-Phenyl, 4.6 mm x 25 cm. Eluant A: 10% acetonitrile, 90% buffer (0.2% triethylamine, 0.25% H_3PO_4). Eluant B: 60% acetonitrile, 40% buffer (0.2% triethylamine, 0.25% H_3PO_4). Gradient profile at 1 mL/min: initialize 100% A, gradient to 80% A, 20% B over 5 minutes, hold 5 minutes, gradient to 100% B over 20 minutes, gradient to 100% A over 5 minutes, hold 20 minutes. Sample preparation: 0.5 - 1.0 g of reaction mixture diluted to 25 mL in acetonitrile - buffer. Hold at ambient temperature about 30 minutes until the purple color of the copper complex is discharged. The desired glycopeptide alkylation product elutes at 16-18 minutes, the starting glycopeptide nucleus at 3-4 minutes, the site N^6 (monosugar) alkylation product at 18-19 minutes, the site N^1 (methyl leucine) alkylation product at 19-21 minutes, dialkylated impurities at 24-26 minutes, and aldehyde at 35-36 minutes. *In situ* yield is determined by correlation to standards prepared with a reference sample of the product.

CLAIMS

1. In a process for reductively alkylating an amine-containing glycopeptide antibiotic, which process comprises reacting the glycopeptide antibiotic with an aldehyde or ketone in the presence of a reducing agent, the improvement which comprises employing pyridine·borane as reducing agent.

2. A process of Claim 1 conducted in methanol at a temperature of from 58-63°.

3. A process of Claim 1 or 2 in which the glycopeptide antibiotic, aldehyde or ketone, and a portion of the reducing agent are mixed together at the same time, and one or more additional portions of reducing agent are added thereafter.

4. A process of any of Claims 1-3 in which a source of soluble copper is additionally supplied.

5. A process of any of Claims 1-4 in which the glycopeptide antibiotic is vancomycin, A82846A, A82846B, A82846C, or orienticin A.

6. A process of any of Claims 1-5 in which the glycopeptide antibiotic is A82846B.

7. A process of any of Claims 1-6 in which the ketone or aldehyde is 4'-chloro-4-biphenylcarboxaldehyde.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21126

A. CLASSIFICATI N OF SUBJECT MATTER
IPC(6) :A01N 37/18; 38/00, 38/14, 38/16
US CL :514/2, 8, 9; 530/322, 345
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/2, 8, 9; 530/322, 345

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, MEDLINE, SCISEARCH, EMBASE, WPIDS, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEE et al. Efficient coupling of glycopeptides to proteins with a heterobifunctional reagent. Biochemistry. 1989, Vol. 28, pages 1856-61, see especially page 1858, left column.	1-7
Y	WONG et al. Pyridine borane as a reducing agent for proteins. Analytical Biochemistry. 1984, Vol. 139, No. 58-67, pages 58-67, see p. 65.	1-7
Y	US 4,877,450 A (BRASCH) 31 October 1989, col. 3, lines 10-30.	4

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21126

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	COOPER et al. Reductive alkylation of glycopeptide antibiotics: synthesis and antibacterial activity. J. Antibiotics. June 1996, Vol. 49, No. 6, pages 575-581, see page 476.	1-7
Y	NAGARAJAN et al. Synthesis and antibacterial evaluation of N-alkyl vancomycins. J. Antibiotics. January 1989, Vol. 42, No. 1, pages 63-72, see page 63.	1-7
Y	TATTANAHALLI et al. The synthesis of netilmicin via complexing of vicinal and non-vicinal amino-hydroxyl group pairs with divalent transition-metal cations. Carbohydrate Research. 1984, Vol. 130, pages 243-249, see page 244.	1-7